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RESEARCH PAPER

Biodiversity of freshwater ciliates (Protista, Ciliophora) in the Lake Weishan Wetland, China: the state of the art

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Abstract

Ciliates are core components of the structure of and function of aquatic microbial food webs. They play an essential role in the energy flow and material circulation within aquatic ecosystems. However, studies on the taxonomy and biodiversity of freshwater ciliates, especially those in wetlands in China are limited. To address this issue, a project to investigate the freshwater ciliates of the Lake Weishan Wetland, Shandong Province, commenced in 2019. Here, we summarize our findings to date on the diversity of ciliates. A total of 187 ciliate species have been found, 94 of which are identified to species-level, 87 to genus-level, and six to family-level. These species show a high morphological diversity and represent five classes, i.e., Heterotrichea, Litostomatea, Prostomatea, Oligohymenophorea, and Spirotrichea. The largest number of species documented are oligohymenophoreans. A comprehensive database of these ciliates, including morphological data, gene sequences, microscope slide specimens and a DNA bank, has been established. In the present study, we provide an annotated checklist of retrieved ciliates as well as information on the sequences of published species. Most of these species are recorded in China for the first time and more than 20% are tentatively identified as new to science. Additionally, an investigation of environmental DNA revealed that the ciliate species diversity in Lake Weishan Wetland is higher than previously supposed.

Keywords Environmental DNA · Protozoa · Species diversity · Taxonomy

Introduction

Ciliates (phylum Ciliophora Doflein, 1901) are a large monophyletic group of unicellular eukaryotes that are the subject of investigation in numerous biological disciplines, such as systematics, evolution, ecology, and cytology, due to their unique cellular structure, functional ecology and life cycles (Gao et al. 2016; Huang et al. 2021; Liu et al. 2021; Song et al. 2021a; Wang et al. 2021a; Zhao et al. 2020). Species of genera, such as *Tetrahymena*, *Paramecium*, and *Stentor*, have been successfully cultured in the laboratory as model organisms to investigate a wide range of biological processes and issues (Sheng et al. 2021; Slabodnick et al. 2017). Nevertheless, knowledge and understanding of ciliate taxonomy and biodiversity, which are important branches of systematics and underpin numerous related disciplines, are limited and it has been estimated that 83–89% of free-living ciliate species have yet to be discovered (Foissner et al. 2008a, b).

In China, the study of ciliates began in 1925 when Wang reported dozens of species (Wang 1925). From then to the

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Special topic: Ciliatology.

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1990s, researchers investigated ciliates from various freshwater habitats in more than 20 provinces in China (Institute of Hydrobiology Chinese Academy of Sciences 1973; Shen 1998; Shen and Gu 2016). However, due to technical limitations, key morphological data, including descriptions of taxonomically important characters such as the pattern of the ciliature, and clear photomicrographs are lacking for most freshwater species reported during this period. Since the 1990s, studies on the taxonomy and systematics of ciliates have been based on modern techniques and, as a result, significant advances have been made in knowledge and understanding of the biodiversity and evolutionary relationships of ciliates in China. However, these works are highly unbalanced in the sense that most have focused on marine rather than freshwater ciliates. This is exemplified by the extensive faunal surveys of the peninsula of Shandong Province and of the coastline of southern China, carried out by several research groups including the Laboratory of Protozoology at the Ocean University of China (Hu et al. 2019; Song et al. 2003, 2009). These works include diversity data for more than 900 species, supported by detailed descriptions of the morphology, phylogeny, genomics and gene evolution, and ecology of many of these. In contrast, there have been only sporadic studies on ciliates from freshwater habitats. To address this deficiency, it is necessary to conduct long-term systematic studies on freshwater ciliate diversity using modern techniques.

Lake Weishan Wetland (LWW) stretches from south to north in a long narrow strip in the southwestern part of Shandong Province, China, and has a drainage area of 31,700 km^2 (Fig. 1). Because of the effects of natural and artificial factors, it has a variety of ecological and environmental habitats resulting in a rich biodiversity (Sun et al. 2019). In addition, as an important node in the South-North Water Diversion Project in China, the biodiversity and ecosystem health of this area have increasingly been a focus of attention. Therefore, we selected the LWW to carry out a 5-year (2019–2023) project on the diversity of freshwater ciliates.

About 200 ciliate species have been found during the first phase of the project, running from 2019 to 2021. A comprehensive database including morphological information, localities, ecological data, gene sequences, microscope slide specimens and a DNA bank, has been established for these species. The morphological information mainly comprises detailed descriptions, photomicrographs and video sequences of living specimens, images of silver-stained specimens, and hand-drawn illustrations. Here, we provided an annotated checklist of retrieved organisms to show the preliminary findings of our investigation. Most of these species are new records for China and about 40 are new to science. The taxonomy of many species has or will be refined or revised. Several publications have been produced and others are in preparation (for details, see Tables 1, 2, 3, 4, 5, 6 and 7). Furthermore, an investigation of environmental DNA revealed that there is a large undiscovered ciliate diversity in the LWW. Here, we provide an overview of the findings of the project to date.

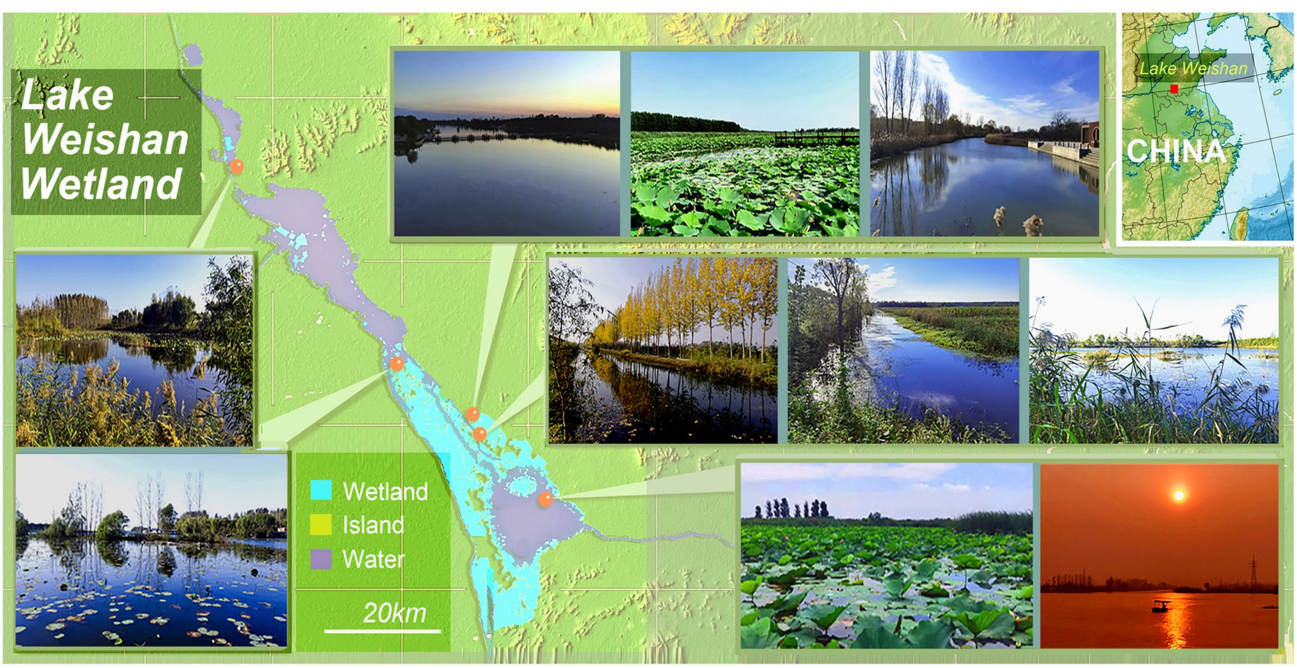

Fig. 1 Sampling sites and habitat characteristics in Lake Weishan Wetland. The insert figure of partial part of China (upper right) indicates the location of Lake Weishan Wetland

Results and discussion

Ecological information (Fig. 2)

From August 2020 to July 2021, a total of 12 water samples and corresponding physicochemical parameters were obtained from LWW. The monthly trends of physicochemical parameters and OTU richness of the planktonic ciliate community are as follows.

Surface water temperature

The surface water temperature decreased from 31.7 to 3.0 °C during the months August to December, and then increased from 3.0 to 29.2 °C during the months January to June (Fig. 2A).

Table 1 Taxa of heterotrichs investigated in the present study

Family	Genus	Species	Details on sampling	Publication	GenBank Acc. No.
Blepharismidae	*Blepharisma*	*Blepharisma penardi*	Lake	–	–
		Blepharisma undulans	Ditch	–	–
		Blepharisma sp. (undescr. sp.?)	Lake	–	–
Climacostomidae	*Climacostomum*	*Climacostomum virens*	Lake	–	–
Condylostomatidae	*Condylostomides*	*Condylostomides coeruleus*	River	–	–
	Linostomella	*Linostomella pseudovorticella*	Channel	–	–
Spirostomidae	*Spirostomum*	*Spirostomum caudatum*	River	Chi et al. (2022a)	OK274292; OM127346; OM127339
Stentoridae	*Stentor*	*Stentor coeruleus*	Fish pond	–	–
		Stentor roeselli	Fish pond	–	–
		Stentor sp.	Lake	–	–

–, data not available

undescr. sp., undescribed species (possibly new species); GenBank Acc. No., GenBank Accession Number

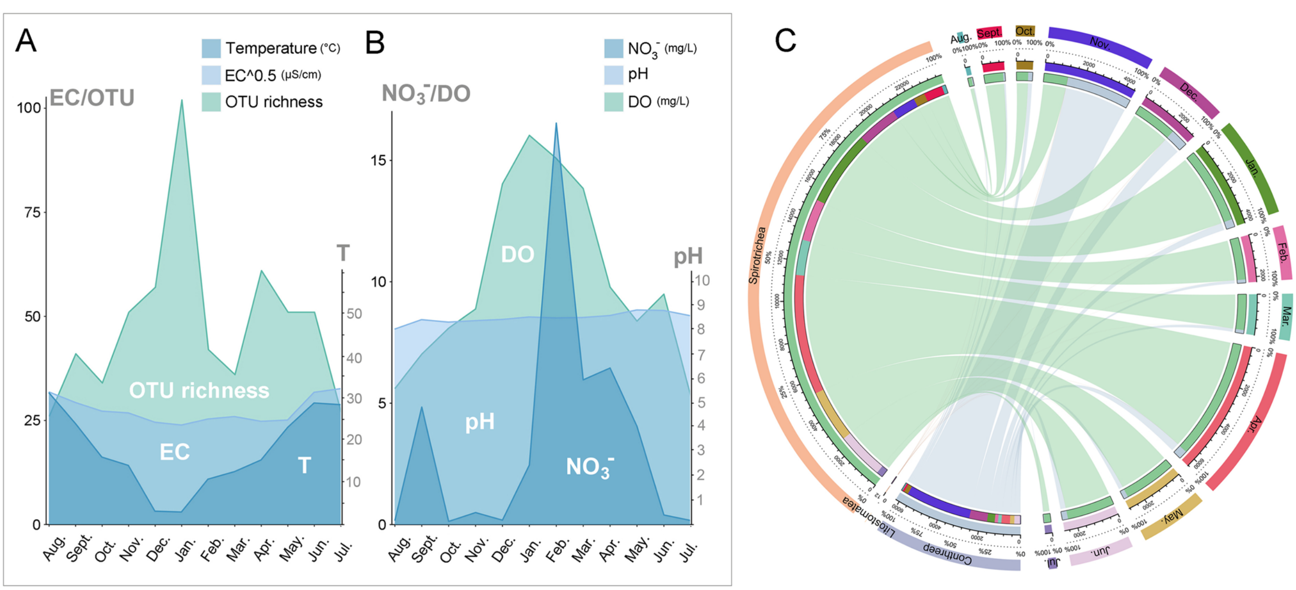

Fig. 2 **A** The variations of surface water temperature, electrical conductivity (EC) and OTU richness of Ciliophora (plankton community) from August 2020 to July 2021. **B** The variations of nitrate (NO_3^-), pH and dissolved oxygen concentration (DO) from August 2020 to July 2021. **C** Temporal variations in the plankton community composition of Ciliophora based on the rank of order from August 2020 to July 2021

Electrical conductivity (EC)

The EC declined rapidly from 1015 to 571 µS/cm from August to January but increased gradually from 571 to 673 µS/cm during January to March, and then increased rapidly from 673 to 1065 µS/cm during May to July (Fig. 2A).

Dissolved oxygen concentration (DO)

The DO increased from 5.06 to 16.03 mg/L during the months August to January and then decreased from 16.03 to 5.29 mg/L during the months January to July (Fig. 2B).

pH

The pH increased from 8.05 in August to 8.44 in September, remained almost stable from September to April, and then increased slightly in May (8.84) and June (8.82). The minima and maxima of pH were in August (8.05) and May (8.84), respectively (Fig. 2B).

Nitrate (NO_3^-)

The NO_3^- concentration increased from 0.17 to 4.85 mg/L from August to September and then declined to 0.14 mg/L in October. It remained at a low level from October to December, increased from 0.19 to 16.55 mg/L during December to February, and then decreased again from 16.55 to 0.18 mg/L during February to July (Fig. 2B).

Operational taxonomic units (OTUs)

In total, 30,076 high-quality sequences of Ciliophora (plankton community) were clustered into 273 OTUs at the 97% similarity level and were assigned to 31 genera, nine families, three orders and one class based on the PR^2 database (Supplementary Table S1). The OTU richness exhibited a negative correlation with temperature ($r = -0.63$, $p < 0.05$) and EC ($r = -0.60$, $p < 0.05$) but was positively correlated with DO ($r = 0.62$, $p < 0.05$) (Fig. 2). The OTU richness increased from 26 to 102 during August to January, declined to 36 in March, increased to 61 in April and then decreased again to 27 during April to July (Fig. 2A).

Based on the sequence data, the planktonic ciliate community was dominated by the Spirotrichea Bütschli, 1889 (79.40%) and also included CONthreeP (20.56%) and Litostomatea Small & Lynn, 1981 (0.04%) from August 2020 to July 2021 (Fig. 2C). Spirotrichea were dominant in all months except November, when CONthreeP (Colpodea, Oligohymenophorea, Nassophorea, Phyllopharyngea, Plagiopylea and Prostomatea) was the most representative group. Litostomatea appeared in only a few months (Fig. 2C). *Tintinnidium* was the most abundant genus, accounting for 30.34% of rRNA gene relative abundance in the ciliate communities, followed by *Halteria* (13.33%).

Overview of species diversity based on morphological observations (Fig. 3)

A total of 187 ciliate morphospecies have been found including 94 identified to species-level, 87 to genus-level, and six species to family-level. The class Oligohymenophorea de Puytorac et al., 1974 was represented by the largest number of species (82), followed by Litostomatea (49), Spirotrichea (37), Heterotrichea Stein, 1859 (10), and Prostomatea Schewiakoff, 1896 (9). The two top-ranked groups at the order-level were Sessilida Kahl, 1933 and Haptorida Corliss, 1974 sensu Lynn (2008) with 49 and 31 species, respectively, while Cyclotrichiida Jankowski, 1980 and Prostomatida Schewiakoff, 1896 only had one species each. At the family-level, Vorticellidae Ehrenberg, 1838 and Epistylididae Kahl, 1933 had the largest number of species, with 18 of each. Thirteen articles have been published with details of these findings (Chi et al. 2021b, 2022a, b; Li et al. 2022; Wang et al. 2021d, 2022b, c; Wu et al. 2021b, c, 2022b, c; Zhang et al. 2022a, b) and several others are in preparation.

Species diversity of heterotrichs (Fig. 4; Table 1)

Background

The class Heterotrichea is considered to be a primitive group within the Ciliophora. Heterotricheans are generally characterized by their large body size, somatic dikinetids associated with postciliodesmata, and an oral apparatus comprising a paroral membrane and an adoral zone of membranelles (Chi et al. 2021a). Due to their large size and omnivorous mode of nutrition, they usually feed on various organisms, from bacteria to small metazoans and microalgae (Foissner and Berger 1996). According to the most recent classification of heterotrichs (Shazib et al. 2014), the class Heterotrichea contains ten families and 58 genera. In China, biodiversity studies on heterotrichs were scarce until the last decade of the twentieth century, and few freshwater species have been systematically investigated (Chi et al. 2020; Hu et al. 2019; Song et al. 2009; Yan et al. 2015, 2016).

Results and remarks

To date, ten species belonging to five families and six genera have been investigated in detail using morphological and

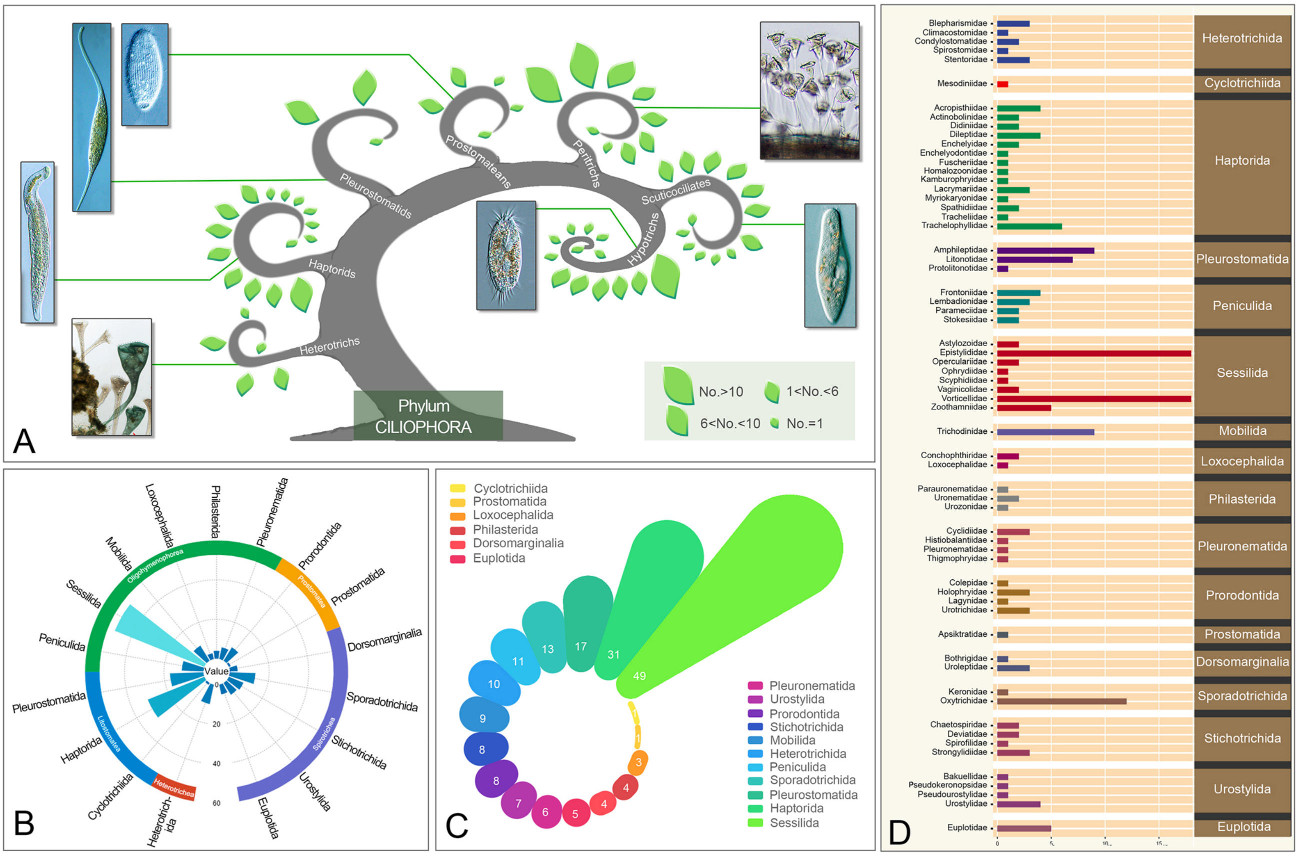

Fig. 3 The species distributions of the investigated groups. **A** The investigated groups. The leaves represent the families included in each group, and the different sizes of leaves represent different values of species number in each family. **B**, **C** Species distributions at order-level. **D** species distributions at family-level

molecular methods (Fig. 4; Table 1). Details of one species, i.e., *Spirostomum caudatum* (Müller, 1786) Delphy, 1939 have been published (Chi et al. 2022a). One species of *Blepharisma* is suspected as new based on comparisons with its congeners. In addition, *Condylostomides coeruleus* Foissner, 2016, which has long been regarded as a biogeographically restricted flagship species and hitherto known only from North America (Hines et al. 2020), was recovered from LWW.

Species biodiversity of haptorids (Fig. 5A–H, J–L; Table 2)

Background

Haptorids are a highly diverse group with more than 1000 nominal species. They show significant variation in size and shape, whereas typical haptorids have an apically or laterally located mouth (Kahl 1930; Lynn 2008). They are raptorial ciliates that feed on flagellates, other ciliates, and even metazoans, such as rotifers and nematodes (Foissner et al. 1995, 1999; Lynn 2008). Although the classification of haptorids has been modified dramatically during the past half-century, taxonomic chaos prevails in much of this group (Huang et al. 2018; Strüder-Kypke et al. 2006; Vd'ačný and Foissner 2013; Vd'ačný and Rajter 2014; Vd'ačný et al. 2011). Recent studies have shown that the diversity of haptorids is much greater than had been previously assumed (Foissner and Oertel 2009; Kwon et al. 2014; Vd'ačný and Foissner 2012). Hitherto, more than 50 species from freshwater habitats, and fewer than 30 species from marine and brackish habitats, have been recorded in China since Wang (1925) described 11 haptorids from Nanjing (Chiang et al. 1983; Hu et al. 2019; Jiang et al. 2021; Shen 1998; Song et al. 2009). However, data, such as photomicrographs, infraciliature, morphometry and details of many key characters, are lacking for many of these species. Consequently, knowledge of the biodiversity of haptorids in China is still in its infancy.

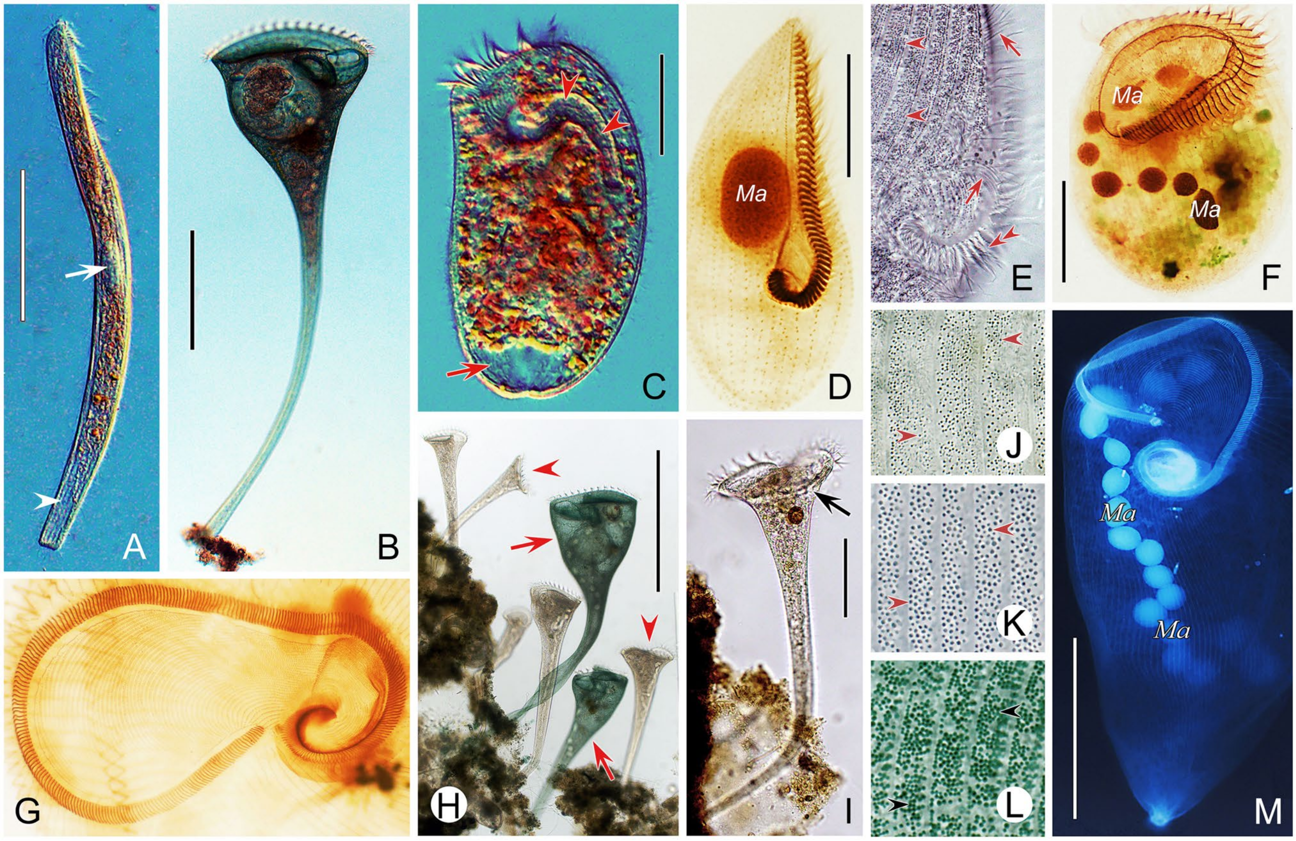

Fig. 4 Selected heterotrichous ciliates found in Lake Weishan Wetland (original). **A** *Spirostomum teres*, arrowhead indicates the contractile vacuole, arrow indicates the macronucleus. **B** *Stentor coeruleus*. **C** *Climacostomum virens*, arrowheads indicate the cyrtos, arrow indicates the contractile vacuole. **D**, **E** *Blepharisma* sp., arrowheads indicate the cortical granules, arrows indicate the paroral membrane, double arrowheads indicate the adoral zone of membranelles. **F** *Linostomella pseudovorticella*. **G** Oral infraciliature of *Stentor coeruleus*. **H** *Stentor* spp., arrowheads indicate *Stentor roeselii*, arrows indicate *Stentor coeruleus*. **I** *Stentor roeselii*, arrow indicates the contractile vacuole. **J** Cortical granules (arrowheads) of *Climacostomum virens*. **K** Cortical granules (arrowheads) of *Stentor roeselii*. **L** Cortical granules (arrowheads) of *Stentor coeruleus*. **M** General infraciliature of *Stentor coeruleus* adjusted by the invert function via Photoshop. *Ma* Macronucleus. Scale bars 50 µm (**C**), 100 µm (**A, D, F, I**), 300 µm (**B, H, M**)

Results and remarks

In the past three years, we investigated 31 haptorids belonging to 14 families isolated from the LWW (Table 2). At least ten of these 31 haptorids have been reported from other continents, supporting their wide distribution model. More than ten unidentified species may be new to science. The generic assignments of two unidentified species of Acropisthiidae Foissner& Foissner, 1988 are uncertain and may represent a new genus. In addition to the type species of *Balantidion*, *B. pellucidum* Eberhard, 1862, another species of this previously monotypic genus was described, namely *Balantidion foissneri* Chi et al., 2022. Based on the morphology of these two *Balantidion* species, Chi et al. (2022b) agreed with Foissner et al. (1999) that *Balantidion* should be assigned to the family Acropisthiidae Foissner & Foissner, 1988 rather than Enchelyidae Ehrenberg, 1838 as suggested by Lynn (2008). Details of two insufficiently known species of *Paradileptus* have been published by Chi et al. (2021b). Some rare species, i.e., found only once or twice during the last half-century, were isolated from LWW, such as *Kamburophrys* sp.

Species diversity of pleurostomatids (Fig. 6; Table 3)

Background

The order Pleurostomatida Schewiakoff, 1896 is a group of raptorial ciliates comprising nearly 200 nominal species. Pleurostomatids are characterized by the bilaterally compressed body, the oral region located along the ventral margin and the bristles on the left side (Foissner et al. 1995; Kahl 1931, 1933; Lynn 2008; Song and Wilbert 1989). Since most pleurostomatids share a similar body shape, it is difficult to distinguish them based only on observations

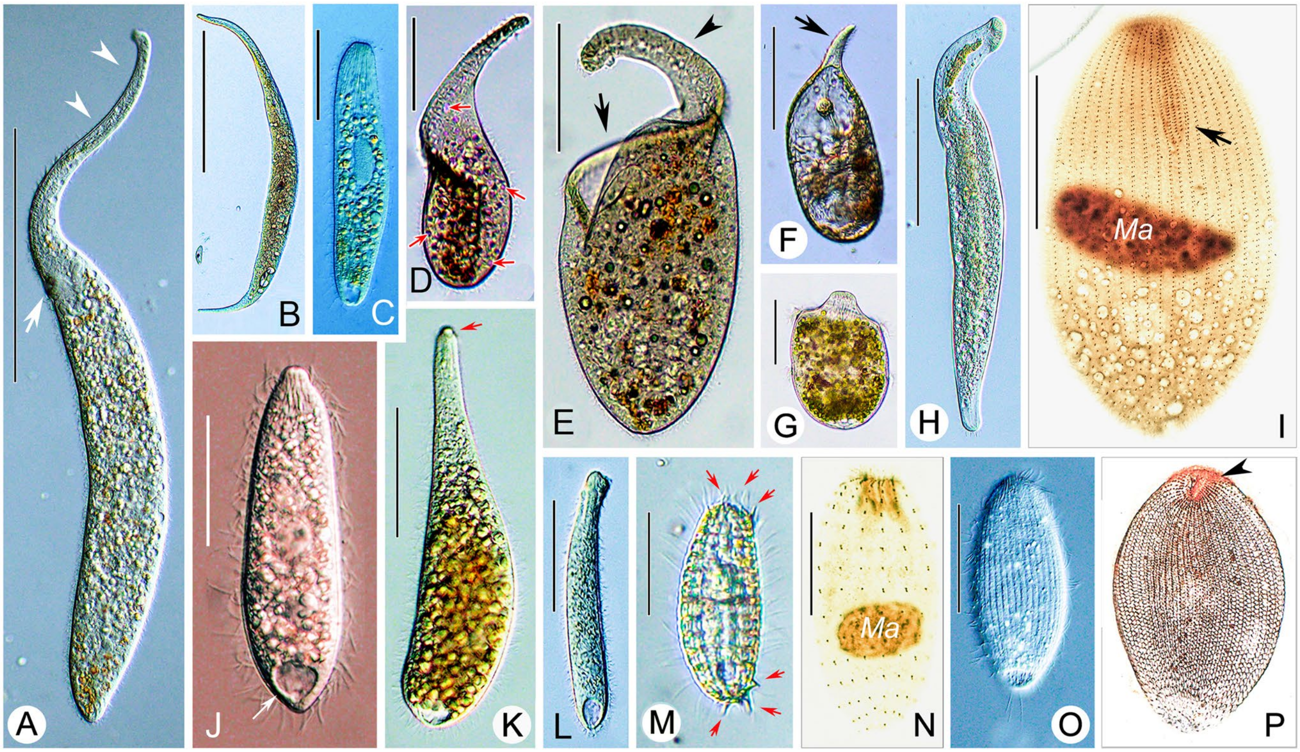

Fig. 5 Selected haptorid (**A–H**, **J–L**) and prorodontid (**I**, **M–P**) ciliates found in Lake Weishan Wetland (original). **A, B** *Pseudomonilicaryon* spp., arrowheads indicate the proboscis, arrow indicates the oral opening. **C**, **J**, **K** *Enchelyotricha* spp., arrow in **J** indicates the contractile vacuole, arrow in **K** indicates the oral bulge. **D**, **E** *Paradileptus* sp., arrows in **D** indicate the contractile vacuoles, arrow in **E** indicates the oral opening, arrowhead in **E** indicates the proboscis. **F** *Trachelius ovum*, arrow indicates the proboscis. **G** *Didinium* sp. **H** *Homalozoon* sp. **I, O, P** *Holophrya* sp. after protargol staining (**I**), in vivo (**O**), and after dry silver nitrate staining (**P**), arrow indicates the brosse, arrowhead indicates the oral opening. **L** *Kamburophrys* sp. **M, N** *Coleps* sp. in vivo (**M**) and after protargol staining (**N**), arrows indicate the spines. Scale bars 60 µm

of cells in vivo. Recent studies based on a combination of morphological and molecular data have revealed a high pleurostomatid diversity and resolved numerous taxonomic confusions (Pan et al. 2020; Vd'ačný et al. 2015; Wu et al. 2021a, 2022a; Zhang et al. 2022a, b). However, phylogenetic relationships within this group are far from being resolved entirely, mainly due to undersampling and the paucity of gene sequence data.

Results and remarks

To date, we have identified 17 pleurostomatids belonging to three families and five genera from the LWW (Table 3). Three new species, *Amphileptus weishanensis* Zhang et al., 2022, *Amphileptus parapleurosigma* Zhang et al., 2022, and *Loxophyllum apochlorelligerum* Zhang et al., 2022, have been described by Zhang et al. (2022a, b). Some poorly known species and genera were also found in the LWW, such as *Litonotus pygmaeus* Vuxanovici, 1959 and *Pseudoamphileptus* Foissner, 1983, all of which lacked reliable morphological data and are being re-investigated based on detailed integrative studies.

Species diversity of prostomateans (Fig. 5I, M–P; Table 4)

Background

Prostomateans are common members of the planktonic community where they can achieve significant abundance and have therefore attracted wide attention from ecologists (Esteban and Fenchel 2021; Foissner et al. 1999; Frantal et al. 2022; Pröschold et al. 2021). In Lynn's (2008) classification, prostomateans comprise two orders, Prostomatida and Prorodontida Corliss, 1974. Prostomatids are characterized by having a genuinely apical oral region, perioral kineties that form obvious paratenes, and neither a brosse nor toxicysts. Prorodontids have an apical or slightly subapical oral region, a brosse and toxicysts. Many prostomateans have not been investigated using modern techniques, even though silver-staining is indispensable for correctly identifying most species (Foissner 2012, 2021; Foissner and Pfister 1997; Foissner et al. 2008a, b; Frantal et al. 2022). In the last decade, the number of new taxa of prostomateans has increased slowly.

Table 2 Taxa of haptorids s. lat. investigated in the present study

Order	Family	Genus	Species	Details on sampling	Publication	GenBank Acc. No.
Cyclotrichiida	Mesodiniidae	*Askenasia*	*Askenasia volvox*	Fish pond	–	–
Haptorida sensu Lynn (2008)	Acropisthiidae	*Balantidion*[1]	*Balantidion foissneri* (undescr. sp.)[2]	Channel	Chi et al. (2022b)	ON158511
			Balantidion pellucidum	Fish pond	–	–
		Undefined[3]	undescr. gen. & sp.?	Fish pond	–	–
		Undefined[3]	undescr. gen. & sp.?	Fish pond	–	–
	Actinobolinidae	*Actinobolina*	*Actinobolina radians*	Fish pond	–	–
			Actinobolina sp. (undescr. sp.)	Fish pond	–	–
	Didiniidae	*Didinium*	*Didinium nasutum*	Lake	–	–
		Monodinium	*Monodinium* sp.	Lake	–	–
	Dileptidae	*Apodileptus*	*Apodileptus visscheri*	Lake	–	–
		Paradileptus	*Paradileptus elephantinus*	Pontoon dock	Chi et al. (2021b)	MZ147012; MZ574467
			Paradileptus conicus	Fish pond	Chi et al. (2021b)	MZ147013; MZ574468
		Pseudomonilicaryon	*Pseudomonilicaryon fraterculum*	Lake	–	–
	Enchelyidae	*Enchelys*	*Enchelys* sp.	Fish pond	–	–
		Papillorhabdos	*Papillorhabdos* sp. (undescr. sp.)	Fish pond	–	–
	Enchelyodontidae	*Enchelydium*	*Enchelydium* sp.	Fish pond	–	–
	Fuscheriidae	*Fuscheria*	*Fuscheria* sp. (undescr. sp.?)	Lake	–	–
	Homalozoonidae	*Homalozoon*	*Homalozoon vermiculare*	River	–	–
	Kamburophryidae	*Kamburophrys*	*Kamburophrys* sp.	Ditch	–	–
	Lacrymariidae	*Lacrymaria*	*Lacrymaria olor*	Fish pond	–	–
			Lacrymaria sp. 1 (undescr. sp.?)	Lake	–	–
			Lacrymaria sp. 2 (undescr. sp.?)	Lake	–	–
	Myriokaryonidae	*Myriokaryon*	*Myriokaryon lieberkuehnii*	Lake	–	–
	Spathidiidae	*Neobryophyllum*	*Neobryophyllum* sp. (undescr. sp.?)	Fish pond	–	–
		Spathidium	*Spathidium polymophum*?	Fish pond	–	–
	Tracheliidae	*Trachelius*	*Trachelius ovum*	Ditch	–	–
	Trachelophyllidae	*Enchelyotricha*	*Enchelyotricha* sp. 1 (undescr. sp.?)	Lake	–	–
			Enchelyotricha sp. 2 (undescr. sp.?)	Lake	–	–
			Enchelyotricha sp. 3	Channel	–	–
		Foissnerides	*Foissnerides* sp.	Fish pond	–	–
		Lagynophrya	*Lagynophrya* sp. (undescr. sp.?)	River	–	–
		Trachelophyllum	*Trachelophyllum brachypharynx*	Lake	–	–

undescr. sp. undescribed species (may be new species), *GenBank Acc. No.* GenBank Accession Number

[1]Family assignation according to Chi et al. 2022a, b

[2]New published species

[3]Same genus

–, data not available

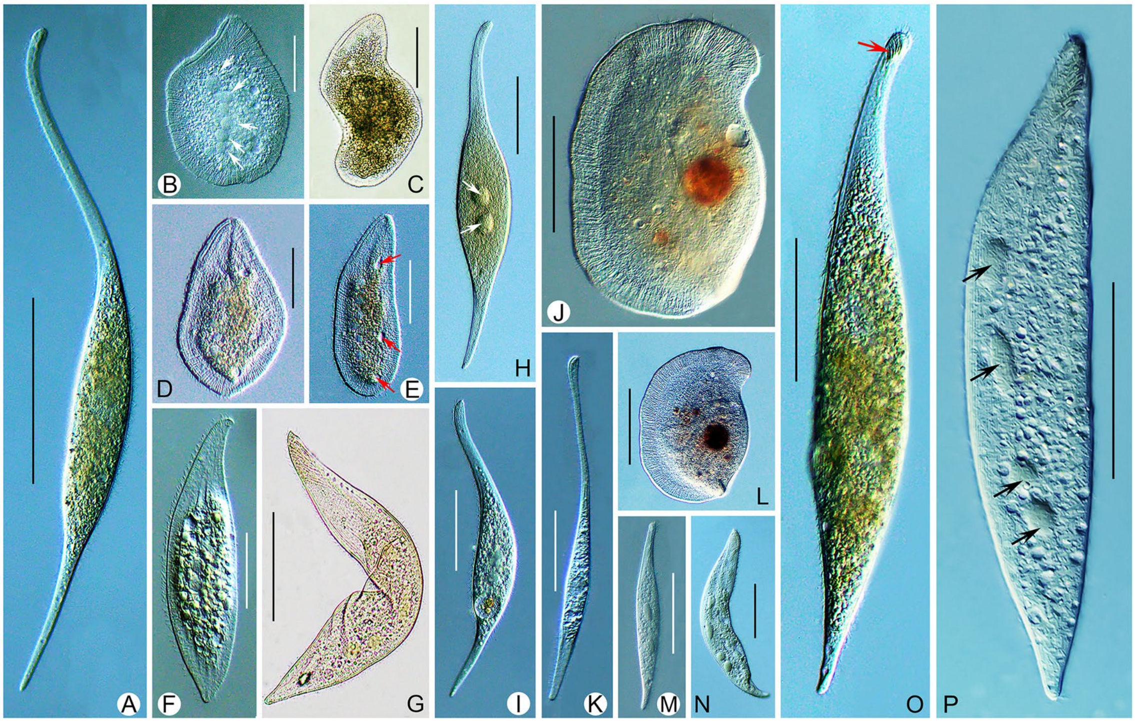

Fig. 6 Selected pleurostomatid ciliates found in Lake Weishan Wetland. **A** *Amphileptus* sp. **B–E** *Kentrophyllum* spp., white arrows indicate the macronuclear nodules, red arrows indicate the contractile vacuoles. **F** *Loxophyllum* sp. **G, N, P** *Amphileptus weishanensis*, arrows in (**P**) indicate the macronuclear nodules. **H, I** *Amphileptus* sp., white arrows indicate the macronuclear nodules. **J, L** *Protolitonotus* sp. **K** *Litonotus* sp. **M** *Amphileptus parapleurosigma*. **O** *Amphileptus* sp., red arrow marks the extrusomes. Scale bars 100 µm (**A, H, I, J, L–N**), 75 µm (**B–F**), 200 µm (**G, P**), 50 µm (**K**), 60 µm (**O**)

However, the findings of Jiang et al. (2021, 2022) suggest that this paucity of species is likely due to undersampling.

Results and remarks

We have investigated nine prostomateans representing five families and six genera (Table 4) and extracted and stored DNA from each to acquire molecular data for future phylogenetic analyses. No findings of our studies on prostomateans have yet been published and investigations of several isolates are ongoing. Nevertheless, new morphological and molecular data are expected to become available for members of the poorly known genera *Lagynus*, *Bursellopsis* and *Apsiktrata*. Up to now, detailed morphological and molecular data are scarce for prostomateans. Molecular data (SSU rDNA) are available for only two prostomatids, i.e., *Metacystis similis* Zhang et al., 2015 and *Foissnerophrys alveolata* Jiang et al., 2021. Phylogenetic analyses of these two questioned the systematics of Prostomatida since they do not form a clade, i.e., *Metacystis similis* is sister to Holophryidae within the other order Prorodontida, and *Foissnerophrys alveolata* groups with the plagiopyleans (Jiang et al. 2021; Zhang et al. 2015). Future studies will focus on the systematics of prostomateans.

Species diversity of scuticociliates and peniculids (Fig. 7; Table 5)

Background

Members of the subclasses Scuticociliatia Small, 1967 and Peniculia Fauré-Fremiet in Corliss, 1956 are extremely widespread in freshwater, brackish and marine habitats (Jung 2021; Li et al. 2021; Liu et al. 2022; Poláková et al. 2021; Serra et al. 2021a, b; Sun et al. 2021; Xu et al. 2018). In these groups, the oral apparatus is generally composed of three membranelles (scuticociliatids) or peniculi (peniculids) and one paroral membrane. The subclass Scuticociliatia comprises more than 300 nominal species. Scuticociliates usually swim fast and are small in size (length about 15–50 µm). While most are free-living and tend to multiply rapidly in eutrophic waters, others are parasitic or symbiotic on the gills, in the blood, or on the body surface of aquatic animals, causing damage to various physiological functions of the host (Song et al. 2003). In contrast, Peniculia is a relatively small subclass (about 100 species reported), with members of the genera *Paramecium* Müller, 1773 and *Frontonia* Ehrenberg, 1833 being the

Table 3 Taxa of pleurostomatids investigated in the present study

Family	Genus	Species	Details on sampling	Publication	GenBank Acc. No.
Amphileptidae	*Amphileptus*	*Amphileptus parapleurosigma*[1]	Wetland	Zhang et al. (2022a)	MW784241
		Amphileptus weishanensis[1]	Port	Zhang et al. (2022a)	MW784242
		Amphileptus sp. 1 (undescr. sp.)[2]	Port	–	OL828281
		Amphileptus sp. 2 (undescr. sp.)[2]	Fish pond	–	OL828282
		Amphileptus sp. 3 (undescr. sp.)[2]	Ditch	–	OL828283
		Amphileptus sp. 4	Wetland	–	–
		Amphileptus sp. 5	Fish pond	–	–
		Amphileptus sp. 6	Fish pond	–	–
	Pseudoamphileptus	*Pseudoamphileptus* sp.	Host: *Cyprinus carpio*; fish pond	–	–
Litonotidae	*Litonotus*	*Litonotus pygmaeus*	Wetland	–	–
		Litonotus sp. 1	Fish pond	–	–
		Litonotus sp. 2	Lake	–	–
		Litonotus sp. 3	Fish pond	–	–
	Loxophyllum	*Loxophyllum apochlorelligerum*[1]	Wetland	Zhang et al. (2022b)	MW414675
		Loxophyllum sp. 1	Wetland	–	–
		Loxophyllum sp. 2	Wetland	–	–
Protolitonotidae	*Protolitonotus*	*Protolitonotus* sp. 1	Lake	–	–

undescr. sp. undescribed species (may be new species), *GenBank Acc. No.* GenBank Accession Number

[1]New published family or genus or species

[2]Have been submitted for publication

–, data not available

Table 4 Taxa of prostomateans investigated in the present study (no publication and no molecular data of these prostomateans online so far)

Order	Family	Genus	Species	Details on sampling
Prorodontida	Colepidae	*Coleps*	*Coleps elongatus*	Lake
	Holophryidae	*Holophrya*	*Holophrya discolor*	An anoxic pool
			Holophrya teres	Ditch
			Holophrya sp.	Lake
	Lagynidae	*Lagynus*	*Lagynus* sp.	An anoxic pool
	Urotrichidae	*Bursellopsis*	*Bursellopsis* sp.	Lake
		Urotricha	*Urotricha valida*	Fish pond
			Urotricha sp.	Lake
Prostomatida	Apsiktratidae	*Apsiktrata*	*Apsiktrata* sp.	An anoxic pool

main contributors to peniculid species diversity. Peniculids usually have a larger body size (length over 50 µm) than scuticociliates, and the majority are free-living members either of the plankton or the benthos.

Results and remarks

In the present project, 13 species of scuticociliates and 11 species of peniculids have been isolated from LWW (Table 5). Most of these isolates are free-living, the exceptions being four scuticociliates (three as symbionts of molluscs and one as a facultative parasite of crabs). Six species were recognized as new species (unpublished, in preparation for publication), i.e., two species of *Frontonia* Ehrenberg, 1833, one species of *Glauconema* Thompson, 1966, one species of *Marituja* Gajewskaja, 1928, one species of *Myxophyllum* Raabe, 1934, and one species of *Pleuronema* Dujardin, 1841.

Species diversity of peritrichs (Figs. 8, 9; Table 6)

Background

The subclass Peritrichia Stein, 1859 is probably the most speciose subclass in the class Oligohymenophorea, with

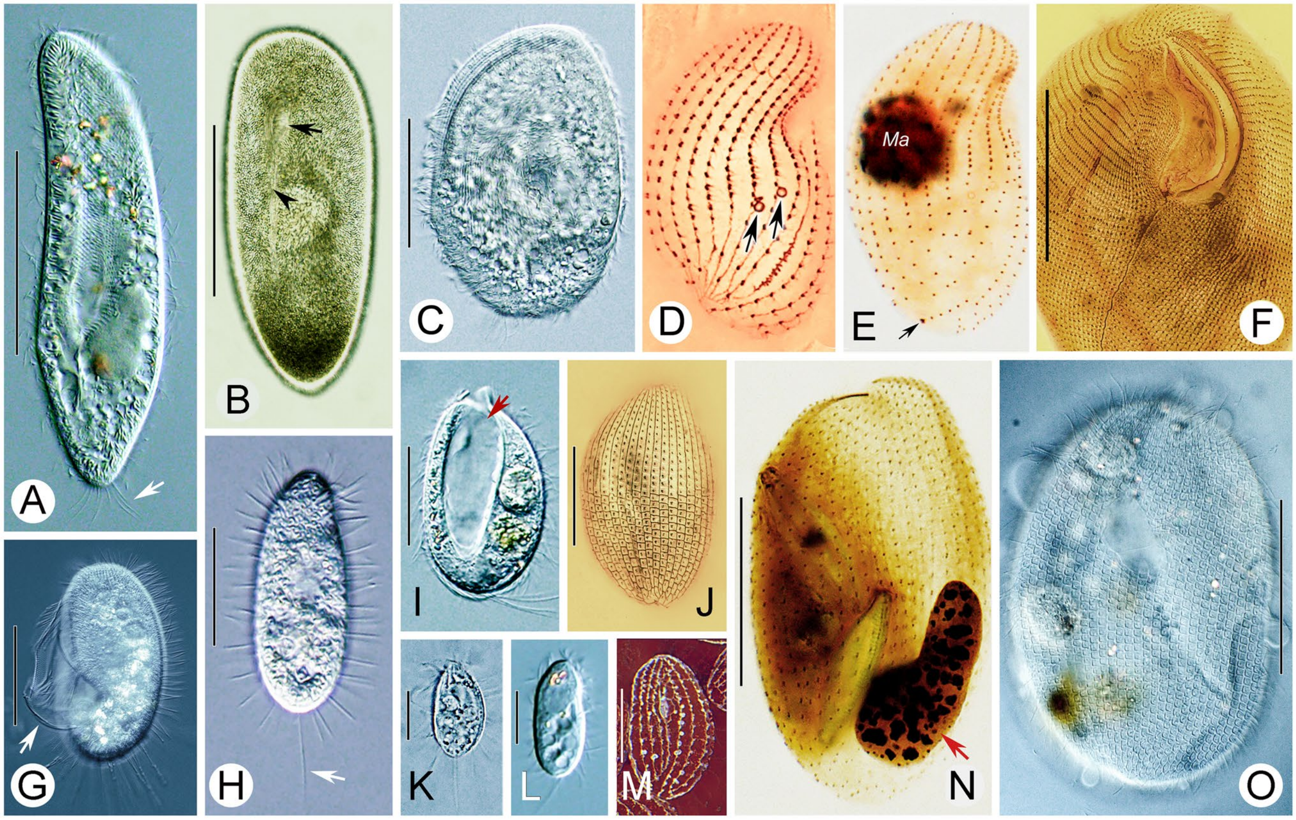

Fig. 7 Selected peniculids and scuticociliates found in Lake Weishan Wetland (original). **A** *Paramecium* sp., arrow indicates the caudal cilia. **B** *Frontonia* sp., arrow indicates the buccal cavity, arrowhead indicates the postoral suture. **C** *Myxophyllum* sp. **D, E** *Glauconema* sp. after silver nitrate (**D**) and protargol staining (**E**), arrows in **D** indicate the contractile vacuole pores, arrow in **E** indicates the caudal complex. **F** *Marituja* sp. **G** *Pleuronema* sp., arrow indicates the paroral membrane. **H** *Dexiotricha granulosa*, arrow indicates the caudal cilium. **I, J, N** *Lembadion lucens* in vivo (**I**), after silver nitrate staining (**J**) and after protargol staining (**N**), arrow in (**I**) indicates the buccal cavity, arrow in (**N**) indicates macronucleus. **K** *Cyclidium* sp. **L, M** *Uronema nigricans*, in vivo (**L**) and after wet silver nitrate staining (**M**, image processed by the invert function via Photoshop). **O** *Lembadion bullinum*. Abbreviation: *Ma*, Macronucleus. Scale bars 100 μm (**A, B, F**), 40 μm (**C**), 30 μm (**G, I, J, N**), 20 μm (**H**), 10 μm (**K–M**), 70 μm (**O**)

more than 1000 nominal species that occupy a wide range of biotopes (Chen et al. 2022; Foissner et al. 1992; Kahl 1935; Wu et al. 2020, 2022b, c). Morphologically, peritrichs are mainly characterized by their conspicuous oral ciliature and vestigial somatic ciliature. The Peritrichia are divided into two orders: Sessilida and Mobilida Kahl, 1933. Most sessilids are sedentary and attach to a substrate via a stalk, a scopula, or a lorica, although some species are free-swimming. Sessilids may be free-living or epibiotic (Gómez et al. 2018; Lynn 2008; Mayén-Estrada and Dias 2021; Safi et al. 2022). Mobilids are characterized by the possession of an adhesive disk, a permanently ciliated trochal band, and all species are epibiotic (Wang et al. 2022b). Early molecular phylogenetic analyses of the Peritrichia based on SSU rDNA sequences indicated that the mobilids have a closer relationship with hymenostomatids than with sessilids (Gong et al. 2006; Zhan et al. 2009). However, this relationship was subsequently found to be an artifact (Zhan et al. 2013). Phylogenomic analyses subsequently provided strong support for the sister relationship between Sessilida and Mobilida and the monophyly of the subclass Peritrichia (Gentekaki et al. 2017; Jiang et al. 2019).

Results and remarks

In the present project, 58 peritrich species comprising 49 sessilids and nine mobilids have been found in the LWW, representing the most significant proportion of ciliate species recorded (Table 6). Several papers have been published that include descriptions of novel species, e.g., *Campanella sinica* Wang et al., 2021, *Epistylis foissneri* Wu et al., 2021, *Epicarchesium sinense* Wu et al., 2021, and *Zoothamnium weishanicum* Wu et al., 2021, or redescriptions of under-researched species (Wang et al. 2021, 2022b, c; Wu et al. 2021b, c). Several unidentified species, such as *Orborhabdostyla* sp. (in preparation for publication), are probably new. Two of these are unidentified species of the family Scyphidiidae which may represent new

Table 5 Taxa of scuticociliates and peniculids investigated in the present study

Order	Family	Genus	Species	Details on sampling	Publication	GenBank Acc. No.
Loxocephalida	Conchophthiridae	*Conchophthirus*	*Conchophthirus* sp. 1	Host: *Sinanodonta woodiana*; wetland	–	–
			Conchophthirus sp. 2	Host: *Cristaria plicata*; wetland	–	–
	Loxocephalidae	*Dexiotricha*	*Dexiotricha granulosa*	wetland	–	–
Philasterida	Parauronematidae	*Glauconema*	*Glauconema* sp. (undescr. sp.)	Wetland	–	–
	Uronematidae	*Uronema*	*Uronema nigricans*	Aquaculture pond	–	–
			Uronema sp.	Wetland	–	–
	Urozonidae	*Urozona*	*Urozona* sp.	Ditch	–	–
Pleuronematida	Cyclidiidae	*Cyclidium*	*Cyclidium* sp. 1	River	–	–
			Cyclidium sp. 2	River	–	–
			Cyclidium sp. 3	Aquaculture pond	–	–
	Histiobalantiidae	*Histiobalantium*	*Histiobalantium* sp.	Wetland	–	–
	Pleuronematidae	*Pleuronema*	*Pleuronema* sp. (undescr. sp.)	Wetland	–	–
	Thigmophryidae	*Myxophyllum*	*Myxophyllum* sp. (undescr. sp.)	Host: *Acusta ravida*; wetland	–	–
Peniculida	Frontoniidae	*Frontonia*	*Frontonia atra*	River	Li et al. (2022)	MZ437443
			Frontonia apoelegans	River	Li et al. (2022)	MZ437442
			Frontonia sp. 1 (undescr. sp.)	Aquaculture pond	–	–
			Frontonia sp. 2	Pond	–	–
	Lembadionidae	*Lembadion*	*Lembadion lucens*	River	–	–
			Lembadion magnum	Wetland	–	–
			Lembadion bullinum	River	–	–
	Parameciidae	*Paramecium*	*Paramecium caudatum*	Pond	–	–
			Paramecium sp.	Pond	–	–
	Stokesiidae	*Marituja*	*Marituja* sp. (undescr. sp.)	River	–	–
		Stokesia	*Stokesia vernalis*	River	Li et al. (2022)	MZ437444

undescr. sp. undescribed species (may be new species), *GenBank Acc. No.* GenBank Accession Number

–, data not available

genera (in preparation for publication). Based on our newly obtained data, a few insufficiently known species will be redescribed or reclassified, including *Epistylis daphniae* Fauré-Fremiet, 1905, *Epistylis lwoffi* Fauré-Fremiet, 1943, *Epistylis pygmaeum* (Ehrenberg, 1838) Foissner et al., 1999, and *Trichodina nigra* Lom, 1960. Some epibiotic sessilids are morphologically similar in vivo to congeners that are free-living. Investigations of these species will provide new insights on the taxonomy and classification of sessilids.

Species diversity of hypotrichs (Fig. 10; Table 7)

Background

Hypotrichs are a large group, i.e., with about 200 genera and 1000 nominal species, of morphologically diverse and ubiquitously distributed ciliates that are widely regarded as the most complex and highly differentiated group within the Ciliophora (Abraham et al. 2021; Jung et al. 2021; Omar et al. 2021; Shao et al. 2020; Vďačný and Foissner 2021; Xu et al. 2020). Most hypotrichs are recognizable by their dorso-ventrally flattened body, conspicuous adoral zone of membranelles, and compound ventral ciliary organelles known as cirri (Berger 1999, 2006, 2011; Curds 1975; Kahl 1932; Small and Lynn 1985; Wang et al. 2021b, c). Since the 1970s, the classification of hypotrichs has undergone several major revisions. According to Shao et al. (2020), Hypotrichia Stein, 1859 includes at least six orders, i.e., Discocephalida Wicklow, 1982, Euplotida Small & Lynn, 1985, Kiitrichida Nozawa, 1941, Stichotrichida Fauré-Fremiet, 1961, Sporadotrichida Fauré-Fremiet, 1961,

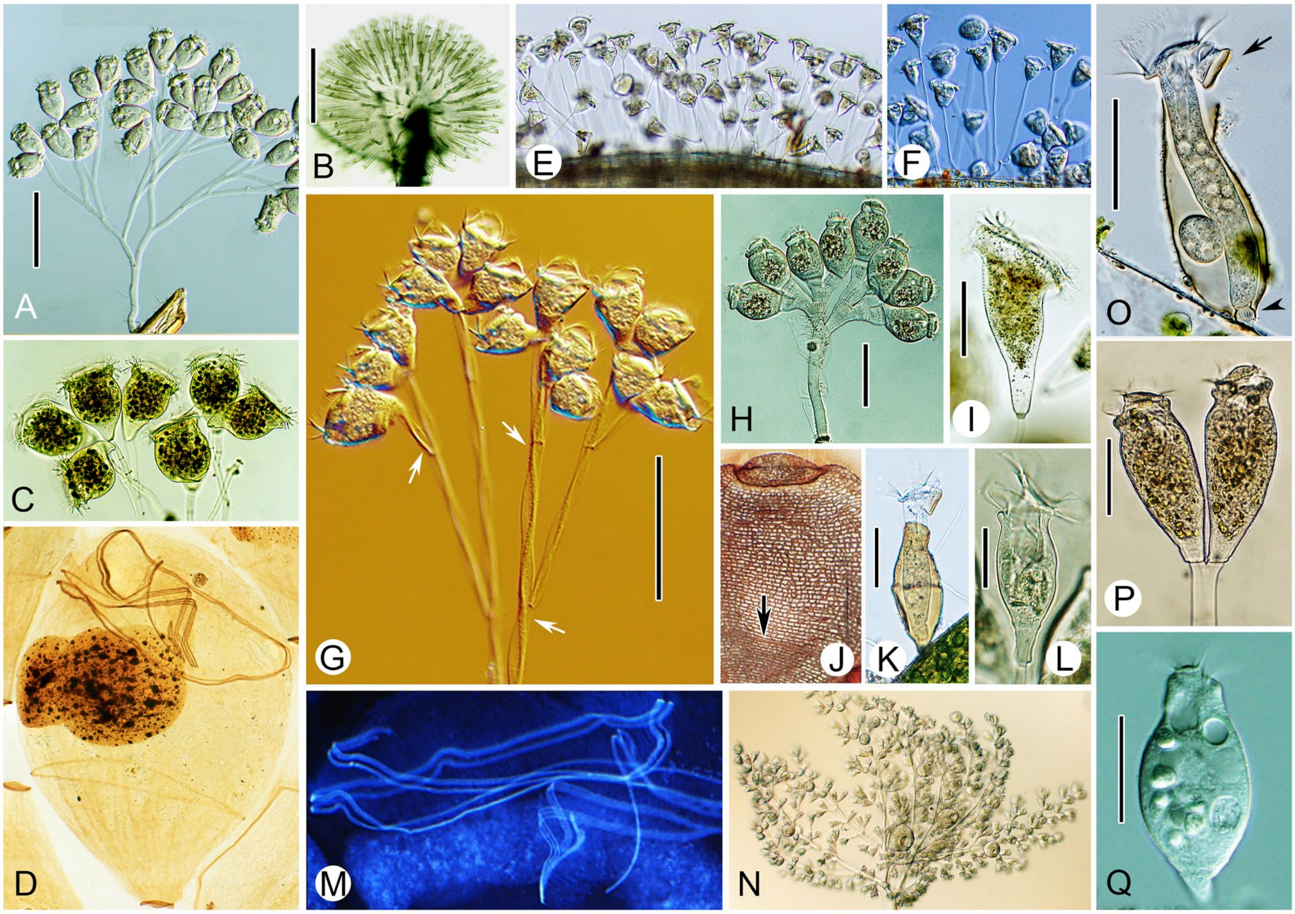

Fig. 8 Selected peritrich ciliates found in Lake Weishan Wetland. **A**, **H**, **P** *Epistylis* spp. **B** *Ophrydium crassicaule*. **C** *Campanella umbellaria*. **D** Protargol-stained zooid of *Epistylis hentscheli*. **E**, **F** *Vorticella* spp. **G** *Epicarchesium sinense*, arrows mark the discontinuous spasmoneme. **I** *Campanella sinica*. **J** Dry silver nitrate-stained zooid of *Epicarchesium granulitum*, arrow marks the trochal band. **K**, **O** *Pyxicola carteri*, arrow marks the operculum, arrowhead marks the tube in lorica base. **L** *Opercularia nutans*. **M** Part of oral ciliature of *Carchesium polypinum* (image processed by the invert function via Photoshop). **N** *Zoothamnium arbuscula*. **Q** *Opercularia* sp. Scale bars 200 μm (**A**, **B**), 150 μm (**G**), 80 μm (**H**, **I**), 30 μm (**K**, **L**), 40 μm (**O**), 70 μm (**P**), 30 μm (**Q**)

and Urostylida Jankowski, 1979. To date, hypotrichs remain one of the most confused groups in terms of their systematics, mainly due to a lack of detailed morphological, ontogenetic, and molecular data for many taxa (Berger 1999, 2006, 2011; Foissner and Berger 2021; Foissner et al. 1991; Paiva 2020; Shao et al. 2020; Zhang et al. 2020).

Results and remarks

In the present study, detailed taxonomic data of 37 hypotrichs (Table 7), including 32 species of hypotrichs s. str. and five euplotids, have been acquired, with morphogenetic information for ten species. Details of the morphology, ontogenesis, and molecular data of two novel species, i.e., *Metaurostylopsis alrasheidi* Song et al., 2020 and *Heterobakuella bergeri* Song et al., 2021, have been published (Song et al. 2020, 2021b). Another two species, *Chaetospira* sp. and *Pseudouroleptus* sp. 1, are also thought to be new species and a novel species of Deviatidae possibly represents a new genus. In addition, more than 15 of these investigated species have also been found in other countries or in different localities within China.

Conclusion, limitations, and prospects

Among the groups involved in the present project, Oligohymenophorea has the largest number of species followed by Spirotrichea. However, this distribution will likely need to be updated or refreshed because investigations of the LWW ciliates are ongoing. The molecular ecological results also imply that there is a higher species diversity than that revealed by morphological studies. Many species found in the LLW have previously been found in other continents,

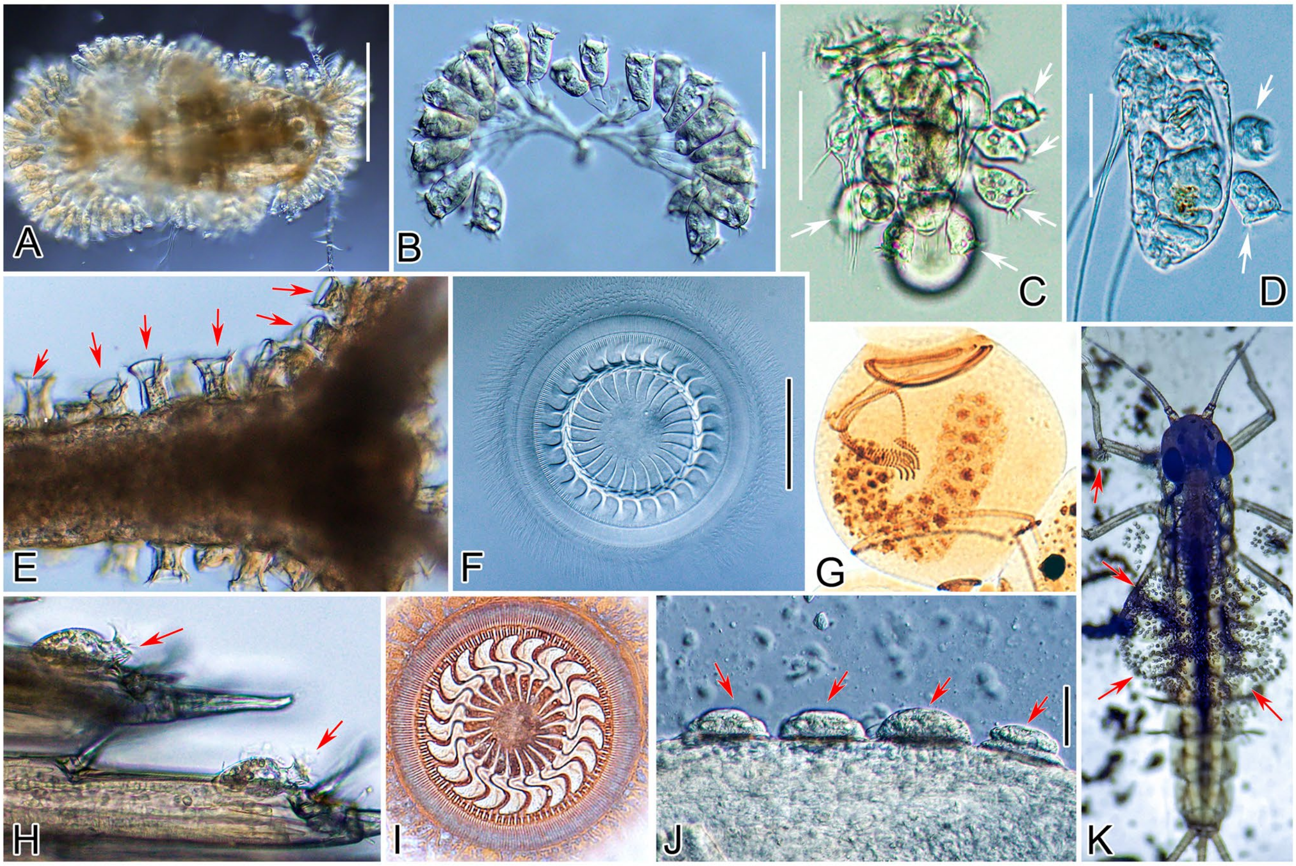

Fig. 9 Selected parasitic/epibiotic peritrich ciliates found in Lake Weishan Wetland (original). **A–D** *Epistylis* spp., arrows indicate the zooids. **E** *Trichodina pediculus* (arrows) on *Hydra* sp. **F** Adhesive disk of *Trichodina nobillis*. **G**, **K** *Carchesium cyclopidarum*, protargol-stained specimen in **G**, arrows in **K** indicate colonies attached to the host. **H** *Lagenophrys* (?) sp. (arrows). **I** Adhesive disk of *Trichodina* sp. (after dry silver nitrate staining). **J** *Trichodina* sp. (arrows) on skin of a fish. Scale bars 0.5 cm in **A**; 100 μm (**B**); 60 μm (**C**, **D**); 20 μm (**F**, **J**)

providing evidence for the cosmopolitan distribution model of ciliates (Finlay 2002; Finlay and Fenchel 2004). Additionally, the comprehensive and detailed taxonomic data obtained in the current project will facilitate accurate species-level identifications for future molecular ecological analyses.

In addition to the taxa mentioned above, a considerable number of ciliates isolated from the LWW have not been well investigated due to a lack of time and expertise, and difficulties in the application of the silver stain. Additionally, our published work has implied some investigated genera/families are non-monophyletic, thus their classification will be revised based on detailed morphological data and molecular analyses. Future work on ciliates in the LWW will focus on: (1) the production of a ciliate atlas including both free-living and parasitic/symbiotic species; (2) the evolutionary relationships of ciliates; (3) revisions of the systematics and classification of selected taxa resulting from the taxonomic and molecular phylogenetic investigations; (4) the process of succession of dominance by different groups of ciliates.

Materials and methods

Sample collection and morphological methods

Samples comprising water, sediment, mud and/or rotting plants were collected from lakes, rivers, canals, brooks, wetlands, ports, and aquaculture ponds in the LWW, Shandong Province, China (Fig. 1). The samples were transferred to the laboratory of Weishan Wetland Station (34° 38′ 34″ N, 117° 16′ 46″ E) in bottles or tanks and checked under a dissecting microscope. For most species, raw cultures were established in Petri dishes (9 cm diameter) containing in situ water and a nutritional resource, such as grains of rice, wheat or millet, and incubated at room temperature (about 25 °C). Living specimens were investigated using bright field and differential interference

Table 6 Taxa of peritrichs investigated in the present study

Order	Family	Genus	Species	Details on sampling	Publication	GenBank Acc. No.
Sessilida	Astylozoidae	*Hastatella*	*Hastatella* sp.	Aquaculture pond	–	–
		Undefined	undescr. gen. & sp.?	Aquaculture pond	–	–
	Epistylididae	*Campanella*	*Campanella sinica*[1]	Ditch	Wang et al. (2021d)	MW969624; MW969625; MW969627
		Epistylis	*Epistylis anastatica*	Host: cyclopoid copepods; aquaculture pond	–	–
			Epistylis cambari	Host: *Procambarus clarkia*; aquaculture pond	–	–
			Epistylis chlorelligerum	Wetland	–	–
			Epistylis daphniae	Host: *Daphnia* sp; ditch	Wu et al. (2022b)	OP289655
			Epistylis foissneri[1]	Wetland	Wu et al. (2021b)	MW172838
			Epistylis galea	Wetland	–	–
			Epistylis hentscheli	Aquaculture pond	Wu et al. (2021b)	MW172839
			Epistylis lwoffi[2]	Host: *Ctenopharyngodon idella*; aquaculture pond	–	–
			Epistylis pygmaeum	Host: Rotifera; aquaculture pond	Wu et al. (2022b)	OP289656
			Epistylis semiciculus	Host: *Procambarus clarkia*; aquaculture pond	–	–
			Epistylis tubificis	Wetland	–	–
			Epistylis vaginula	Wetland	Wu et al. (2021b)	MW172840
			Epistylis weishanensis[1]	Host: *Gammarus* sp; wetland	Wu et al. (2022b)	OP289654
			Epistylis sp. 1	Host: *Procambarus clarkia*; aquaculture pond	–	–
			Epistylis sp. 2	Wetland	–	–
			Epistylis sp. 3	Wetland	–	–
		Orborhabdostyla	*Orborhabdostyla* sp. (undescr. sp.)[3]	Aquaculture pond	–	–
	Operculariidae	*Opercularia*	*Opercularia nutans*	Float grass in a lake	–	–
		Undefined	Undefined	Aquaculture pond	–	–
	Ophrydiidae	*Ophrydium*	*Ophrydium crassicaule*	Float grass in a lake	Wu et al. (2022c)	OM801561 OM801562 OM801564
	Scyphidiidae	Undefined	Undescr. gen. & sp.[3]	Host: *Ctenopharyngodon idella*; aquaculture pond	–	–
	Vaginicolidae	*Cothurnia*	*Cothurnia* sp.	Fish pond	–	–
		Pyxicola	*Pyxicola carteri*	Fish pond	–	–

Table 6 (continued)

Order	Family	Genus	Species	Details on sampling	Publication	GenBank Acc. No.
	Vorticellidae	*Carchesium*	*Carchesium cyclopidarum*	Ditch	Wang et al. (2022c)	ON158510
			Carchesium polypinum	Fish pond	Wu et al. (2021c)	MZ478146
		Epicarchesium	*Epicarchesium granulatum*	Host: *Procambarus clarkia*; aquaculture pond	–	–
			Epicarchesium sinense[1]	Aquaculture pond	Wu et al. (2021c)	MZ478145
		Pseudovorticella	*Pseudovorticella* sp. 1	Fish pond	–	–
			Pseudovorticella sp. 2	Pond	–	–
			Pseudovorticella sp. 3 (undescr. sp.?)	Ditch	–	–
		Vorticella	*Vorticella* sp. 1	Pond	–	–
			Vorticella sp. 2	Pond	–	–
			Vorticella sp. 3	Fish pond	–	–
			Vorticella sp. 5	Float grass in a lake	–	–
			Vorticella sp. 6	Wetland	–	–
			Vorticella sp. 7	Fish pond	–	–
			Vorticella sp. 8	Wetland	–	–
			Vorticella sp. 9	Ditch	–	–
			Vorticella sp. 10	Port	–	–
			Vorticella sp. 11 (undescr. sp.?)	Float grass a lake	–	–
		Vorticellides	*Vorticellides aquadulcis*	Aquarium	–	–
	Zoothamniidae	*Zoothamnium*	*Zoothamnium arbuscula*	Wetland	Wu et al. (2021c)	MZ478144
			Zoothamnium hentscheli	Wetland	Wu et al. (2021c)	MZ478143
			Zoothamnium procerius	Host: *Procambarus clarkia*; aquaculture pond	–	–
			Zoothamnium weishanicum[1]	Wetland	Wu et al. (2021c)	MZ478142
			Zoothamnium sp.	Wetland	–	–
Mobilida	Trichodinidae	*Trichodina*	*Trichodina acuta*	Host: *Cyprinus carpio*; fish pond	Wang et al. (2022b)	MT982920
			Trichodina funduli	Host: *Cyprinus carpio*; fish pond	–	–
			Trichodina nigra	Host: *Cyprinus carpio*; fish pond	Wang et al. (2022b)	MT982921
			Trichodina nobillis	Host: *Ctenopharyngodon idella*; fish pond	–	–
			Trichodina pediculus	Host: *Hydra* sp.; ditch	–	–
			Trichodina sp. 1	Host: *Channa argus*; fish pond	–	–
			Trichodina sp. 2	Host: *Siniperca chuatsi*; fish pond	–	–
			Trichodina sp. 3	Host: *Anodonta woodiana*; fish pond	–	–
		Trichodinella	*Trichodinella* sp.	Host: *Ctenopharyngodon idella*; fish pond	–	–

undescr. sp. undescribed species (new to science), *GenBank Acc. No.* GenBank Accession Number
[1]New published species

[2]Transferred into genus *Heteropolaria* by Foissner and Schubert (1977), however, reassigned into *Epistylis* by Wu et al. (in preparation)

[3]In preparation for publication

–, data not available

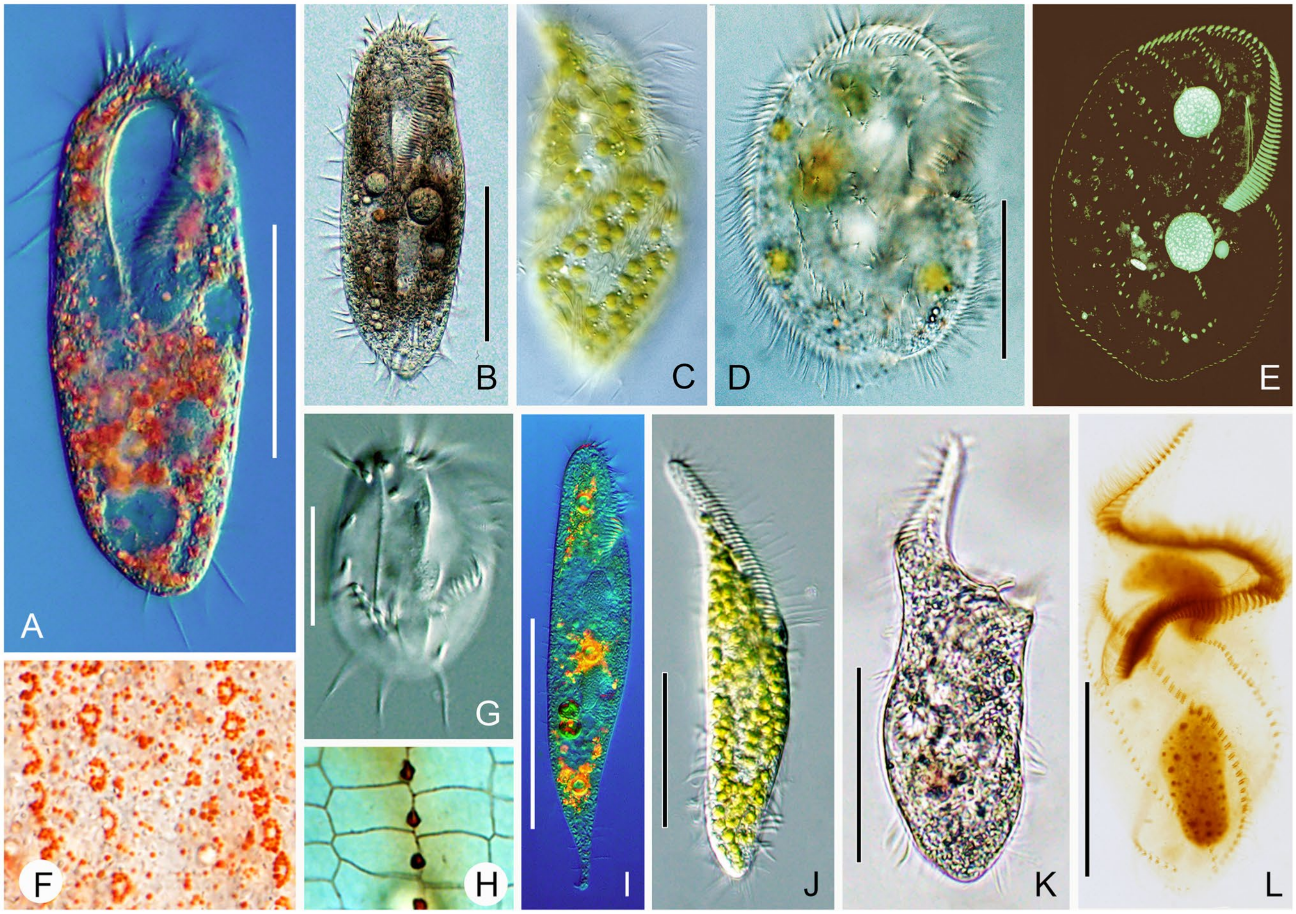

Fig. 10 Selected hypotrichous ciliates found in Lake Weishan Wetland (original). **A** *Cyrtohymena primicirrata*. **B** *Pleurotricha curdsi*. **C**, **J** *Stichotricha* sp. **D**, **E** *Kerona pediculus* in vivo **(D)** and after protargol staining (**E**, image processed by the invert function via Photoshop). **F** Cortical granules of *Pseudokeronopsis erythrina*. **G** *Euplotes muscicola*. **H** Detail of silverline system on dorsal side of *Euplotes* sp. **I** *Urosoma caudata*. **K**, **L** *Chaetospira* sp. in vivo (**K**) and after protargol staining (**L**) Scale bars 50 µm (**A**, **B**, **D**, **I–L**), 20 µm (**G**)

contrast microscopy (Olympus BX53; Olympus Corporation, Tokyo, Japan) at magnifications of 40–1000×. Protargol staining, dry silver nitrate staining, wet silver nitrate staining and silver carbonate staining were performed following Wilbert (1975), Klein (1958), Corliss (1953), and Ma et al. (2003), respectively. Classification mainly follows Lynn (2008). The charts shown in Fig. 3 were made by GENESCLOUD (www.genescloud.cn/).

DNA extraction and PCR amplification, and sequencing

Ciliate cells were isolated and washed several times in double distilled water using glass micropipettes under a dissecting microscope. At least one cell was used for extracting the total genomic DNA using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The DNA polymerase, primers, and programs used in PCR amplification were according to Chi et al. (2020, 2021a, b) and Wang et al. (2021). The fragments were sequenced bidirectionally by the Tsingke Biological Technology Company (Qingdao, China). Sequences of some species have been deposited in the GenBank database (for accession numbers, see Tables 1, 2, 3, 4, 5, 6 and 7). All DNA samples have been coded and stored at the Laboratory of Protozoology at the Ocean University of China.

Environmental and molecular ecological analyses

Taking into consideration that our biodiversity/taxonomic survey was especially lacking data concerning lake planktonic ciliates, we decided to make a preliminary screening of planktonic ciliate biodiversity using high-throughput sequencing. Results were only aimed at providing preliminary data for further study, either involving traditional taxonomic approaches or high-throughput sequencing.

Table 7 Taxa of hypotrichs s. lat. investigated in the present study

Order/group	Family	Genus	Species	Details on sample	Publication	GenBank Acc. No.
Dorsomarginalia	Bothrigidae	*Bothrix*	*Bothrix africana*	Fish pond	–	–
	Uroleptidae	*Uroleptus*	*Uroleptus gallina*	Wetland	–	–
			Uroleptus longicaudatus	Wetland	–	–
			Uroleptus sp.	Wetland	–	–
Sporadotrichida	Keronidae	*Kerona*	*Kerona pediculus*	Host: Hydra sp.; ditch	–	–
	Oxytrichidae	*Cyrtohymena*	*Cyrtohymena primicirrata*	Ditch	–	–
		Monomicrocaryon	*Monomicrocaryon euglenivorum euglenivorum*	Wetland	–	–
		Notohymena	*Notohymena australis*	Ditch	–	–
		Oxytricha	*Oxytricha* sp. 1	Wetland	–	–
			Oxytricha sp. 2	Wetland	–	–
		Paraurostyla	*Paraurostyla weissei*	Wetland	–	–
		Pleurotricha	*Pleurotricha curdsi*	Wetland	–	–
		Pseudostromhidium	*Pseudostromhidium planctonticum*	Fish pond	–	–
		Rubrioxytricha	*Rubrioxytricha haematoplasma*	Wetland	–	–
		Sterkiella	*Sterkiella* sp.	Wetland	–	–
		Stylonychia	*Stylonychia pustulata*	Wetland	–	–
		Urosoma	*Urosoma caudata*	Ditch	–	–
Stichotrichida	Chaetospiridae	*Chaetospira*	*Chaetospira* sp. (undescr. sp.)	Fish pond	–	OM313318; OM313319; OM313320
		Stichotricha	*Stichotricha* sp.	Wetland	–	–
	Deviatidae	*Deviata*	*Deviata polycirrata*	Brook	–	–
		Undefined	undescr. gen. & sp.?	Brook	–	–
	Spirofilidae	*Hypotrichidium*	*Hypotrichidium* sp.	Ditch	–	–
	Strongylidiidae	*Pseudouroleptus*	*Pseudouroleptus* sp. 1 (undescr. sp.)	Fish pond	–	–
			Pseudouroleptus sp. 2	Fish pond	–	–
		Strongylidium	*Strongylidium wuhanense*	Wetland	–	–

Table 7 (continued)

Order/group	Family	Genus	Species	Details on sample	Publication	GenBank Acc. No.
Urostylida	Bakuellidae	*Heterobakuella*[1]	*Heterobakuella bergeri*[1]	Brook	Song et al. (2021b)	MW692986
	Pseudokeronopsidae	*Pseudokeronopsis*	*Pseudokeronopsis erythrina*	Lake	–	–
	Pseudourostylidae	*Diaxonella*	*Diaxonella trimarginata*	Wetland	–	–
	Urostylidae	*Holosticha*	*Holosticha pullaster*	Ditch	–	–
		Neourostylopsis	*Neourostylopsis flava*	Wetland	–	–
		Metaurostylopsis	*Metaurostylopsis alrasheidi*[1]	Wetland	Song et al. (2020)	MT911525
		Pseudourostla	*Pseudourostla cristata*	Ditch	–	–
Euplotida	Euplotidae	*Euplotes*	*Euplotes woodruffi*	Wetland	–	–
			Euplotes muscicola	Fish pond	–	–
			Euplotes sp. 1	Wetland	–	–
			Euplotes sp. 2	Wetland	–	–
			Euplotes sp. 3	Wetland	–	–

undescr. sp undescribed species (may be new species), *GenBank Acc. No.* GenBank Accession Number

[1]New published genus or species

–, data not available

From August 2020 to July 2021, 5 L lake water (from ~ 50 cm below the water surface) was collected from LWW on each sampling occasion for environmental DNA extraction. Dissolved oxygen concentration (DO), electrical conductivity (EC), pH and temperature were measured in situ with a multi-parameter water quality sonde (YSI, USA). The surface water samples were immediately transported to the laboratory and filtered through 0.22 μm pore-size PVDF membranes (Millipore, Ireland) using a vacuum pump until the filter was completely clogged. Then, filters were immediately stored at − 80 °C until DNA extraction. The filtrate was used to measure the nitrate (NO_3^-) concentration by ion chromatography (Thermo Scientific, USA).

Total DNA was extracted in triplicate using the DNeasy PowerWater kit (Qiagen, USA) following the manufacturer's recommendations. Triplicate DNA extractions of each sample were combined and mixed to form a pooled sample. The primers EK-565F (5′-GCAGTTAAAAAGCTCGTAGT-3′) and EK-1134R (5′-TTTAAGTTTCAGCCTTGCG-3′) were used to amplify the hypervariable V4 region of the 18S rDNA (Bower et al. 2004; Simon et al. 2015). The triplicate PCR reactions and cycling parameters of the 18S amplicon were according to Wang et al. (2022). PCR products from triplicate reactions per sample were purified, pooled, and then sequenced on the Illumina MiSeq platform (Majorbio, Shanghai, China).

Paired-end reads were merged with FLASH (Magoč and Salzberg 2011), trimmed with Trimmomatic (Bolger et al. 2014), and quality-filtered by QIIME v1.8 (Caporaso et al. 2010). Chimeric sequences were identified and removed by UCHIME (Edgar et al. 2011). Sequences that occurred only once in the entire data set (singletons) were also excluded. Operational taxonomic units (OTUs) were clustered with 97% sequence similarity cutoff using UPARSE (Edgar 2013). Taxonomic classification of the OTUs was carried out by RDP Classifier algorithm against the PR^2 database (Guillou et al. 2012). Prior to statistical analyses, samples were rarefied to an even sampling depth by random resampling (McMurdie and Holmes 2013). Statistical analyses were conducted using the "vegan" package and visualized by the "ggplot2" package in R v.3.6.3 (Oksanen et al. 2014; Wickham 2016). Temporal variations in the plankton community composition of Ciliophora at the rank of order were analyzed by the "circlize" package.

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Author contributions WBS and HGM conceived and guided the study. YC, BRL, GATZ, TL, ZW, TW, WYS, YFW and YJL conducted the research; YC wrote the content of heterotrichs; BRL wrote the content of haptorids and prostomateans; GATZ wrote the content of pleurostomatids; TL wrote the content of hymenostomatids; ZW, TW wrote the content of peritrichs; WYS wrote the content of hypotrichs; YFW wrote the content of ecology; KASA and AW reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

Data availability Most datasets generated or analyzed during this study are included in this published article (and its supplementary material), and the rest, e.g., DNA, some gene sequences, and morphological details, are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest. Author Alan Warren and Weibo Song are members of the Editorial Board, but they were not involved in the journal's review of, or decision related to, this manuscript.

Animal and human rights statements We declare that all applicable international, national, and or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed and all necessary approvals have been obtained.

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